THE HEXONE BASES OF EGG-WHITE.

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The separation of the hexone bases from the products of the hydrolysis of the proteins of egg-white dates from the work of Siegfried*, communicated by Drechsel†. In 1895, Hedin‡ separated 2·4 gm. arginin from 300 gm. Albumin aus Eiweiss (Grubler), a yield of 0·8%. In 1896, Hedin recognised lysin§; and, a little later, histidin‖ among the products of the dissociation of the same substance.

In 1899, Hausmann¶ determined the amount of diamino nitrogen in egg-albumin prepared by the method of Hofmeister. His figure represents that portion of nitrogen which is precipitable by phosphotungstic acid after the ammonia has been removed by boiling with magnesia. The hydrolysis was carried out with boiling hydrochloric acid. Hausmann found 21·33% of the

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* Ber. xxiv., S. 418, 1891.
† Arch. Anat. u. Phys., S. 271, 1891.
‡ Zeit. physiol. Chem., xxi., S. 163, 1895 6.
§ Zeit. physiol. Chem., xxi., S. 302, 1895-6.
‖ Zeit. physiol. Chem., xxii., S. 191, 1896-7.
¶ Zeit. physiol. Chem., xxvii., S. 95, 1899.

nitrogen precipitable under the conditions of the experiment. This amounted to 3·31% of the weight of the egg-albumin. Kutscher*, who carried out similar determinations, found 27% of the nitrogen present as diamino nitrogen in Eieralbumin free from globulin and ovomucoid. This represented 4·19% of the weight of the albumin. Osborne and Harris† found the basic, *i.e.*, the diamino nitrogen to be 3·3% of the weight of ovalbumin, and 4·16% of the weight of conalbumin, both prepared from hens' eggs. Osborne, Leavenworth, and Brautlecht‡ determined the quantities of the individual hexone bases arginin, histidin, and lysin in ovalbumin and conalbumin. They state that the amount of nitrogen contained in these bases corresponds closely with that precipitated by phosphotungstic acid. They therefore consider that determination of nitrogen precipitated by phosphotungstic acid furnishes a valuable means for controlling the results of base determinations in proteins.

Hugounenq and Galimard§ found 2·5% lysin, 2·14%arginin, and no histidin in the ovalbumin of hen's egg.

Scope of investigation.—The amounts of arginin, histidin, and lysin have been estimated in the products of the hydrolysis of hen's egg-white. The whole egg-white, rather than any separated purified protein, has been employed since egg-white is used for experimental work in nutrition and not purified protein. The isolation of chemically pure proteins from egg-white is difficult, and it is doubtful whether the methods of separation for pure proteins are as yet satisfactory.‖ It is also not proven that the separate proteins of egg-white are bodies of constant and definite constitution.

In certain preliminary attempts the hydrolysis has been performed with 5% sulphuric acid, and, also (by one of us, H.G.C.),

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* Zeit. physiol. Chem., xxxi., S. 215, 1901.

† Journ. Amer. Chem. Soc., xxv., p. 346, 1903.

‡ Amer. Journ. Physiol., xxiii., p. 194, 1908.

§ C.R., T. 143, p. 242, 1906.

‖ Compare Mellanby, Journ. of Physiol., xxxvi., p. 288, 1907.

with pancreatic juice, obtained by the injection of secretin into a dog and activated by enterokinase. These hydrolyses were incomplete, though continued for many hours. A complete hydrolysis has been accomplished with 25% sulphuric acid.

Methods.—The methods of separation devised by Kossel and Kutscher*, with the modifications of Kossel and Patten† and Osborne, Leavenworth and Brautlecht,‡ have been employed. In four out of the five determinations, the hexone bases were first precipitated by phosphotungstic acid.

Hydrolysis.—In the final experiment, 680 c.c. egg-white containing 95·7 gm. solid matter, 89·6 gm. protein, and 12·32 gm. nitrogen were placed in a steam sterilizer at 100°C., with 150 c.c. water and 50 c.c. sulphuric acid, for 7 hours. Then 200 c.c. sulphuric acid were added, and the mixture steamed for 6 hours. It was now heated to the boiling point on a sand-bath with a a reflux condenser, for 69 hours. The volume was made up to 1 litre, and 10 c.c. were removed for the estimation of amide ammonia and melanoidin nitrogen§. The results gave 1·05 gm. nitrogen as ammonia, and 0·224 gm. nitrogen as melanoidin. Previously attempts had been made to carry out the hydrolysis with 5% sulphuric acid. Thus 39·25 gm. dried egg-white (250c.c.) were boiled in 500 c.c. 5% sulphuric acid for 50 hours. The biuret test was positive after 25 hours' ebullition, but at the end it appeared to be negative. Two other experiments, one continued for 17 hours, and the other for 48 hours, were carried out. In these, and also in the tryptic digest the amounts of hexone bases were much too small, while the percentage of nitrogen precipitated by phosphotungstic acid was too large‖. Certain of the final products were contaminated with bodies resembling the

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* Zeit. physiol. Chem., xxxi., S.165, 1901.

† Zeit. physiol. Chem., xxxviii., S.39, 1903.

‡ *Loc. cit.*

§ Osborne and Harris, *loc. cit.*

‖ Nitrogen present as ammonia was expelled before the estimations were made.

dipeptide isolated by Levene and Beatty*. Corroboration was thus obtained of the difficulty in completing the hydrolysis of certain proteins, as noted by Osborne, Leavenworth, and Brautlecht.

Separation of hexone bases by phosphotungstic acid.—The filtered digest was diluted to contain 5 % sulphuric acid. Phosphotungstic acid was added until the 5 litres contained about 5 % reagent. The precipitate that fell, on the further addition of phosphotungstic acid, consisted only of reagent. After standing 72 hours the superfluid was decanted, and the remaining portion separated in the centrifuge. All the separations, with the subsequent washing of precipitates, have been done in the centrifuge. Much labour and time are saved in this way. Many precipitates are driven down hard by 10 minutes' spinning, so that the superfluid can be poured off. Three or four washings then suffice, as the hard, practically dry mass can be suspended in water and thoroughly mixed or heated as required. The solid matter is again separated in the centrifuge.

Separation of arginin from histidin.—The separation of arginin from histidin was, in our hands, difficult. This was probably due to inexperience, as an improvement was noted in each subsequent hydrolysis. It was invariably found that much arginin was precipitated with the histidin in the second silver precipitation of Kossel and Kutscher. This was obvious upon applying Kossel and Patten's process to the histidin portion. Nitrogen determinations showed the presence of a quantity of arginin in the filtrate from mercuric sulphate. Further, it seemed that the mercuric sulphate did not completely precipitate histidin, as frequently four or five precipitates containing histidin separated from the filtrate, if this was allowed to stand two or three weeks.

On employing the precipitation by mercuric sulphate of Kossel & Patten previous to the second silver precipitation of Kossel & Kutscher, as suggested by Osborne, Leavenworth and Brautlecht, no difficulty was experienced in the separation.

* Biochem. Zeitsch. iv., S. 299, 1907.

Estimation of the separated hexone bases.—Two methods have been employed. In the first method the amount of nitrogen present in the final solution is estimated, and the quantity of base calculated from this figure. The bases are isolated as salts only for identification. In the second method the isolated salts are used to calculate the weight of bases.

While little can be said against the practice of calculating the weight of base present in the "purified" fluids from which the salts are crystallised, there is more doubt concerning another practice recently introduced. Jackson and Pearce* estimated the total hexone base from the amount of nitrogen precipitated by phosphotungstic acid, the arginin and histidin from the nitrogen precipitated by silver nitrate and baryta, and the lysin from the nitrogen in the filtrate freed from silver and baryta. Siegfried and Pilz† adopted a somewhat similar practice. The results recorded from our experiments in Table i., show that both these portions (histidin plus arginin, and lysin) contain nitrogen not attributable to these substances.

Results.—A record of two of our experiments is given in Table i. The amounts of nitrogen obtained at the various stages of the separation are stated. The first hydrolysis was performed with 5 % sulphuric acid for 17 hours, and the second with 25 % sulphuric acid for 69 hours. The first separation was conducted according to Kossel & Patten, the second by the modification of Osborne, Leavenworth & Brautlecht. In each case the precipitation with silver and baryta was repeated to throw down completely the arginin.

The percentage of total nitrogen present in each stage enables the two results to be compared. The high figure in the first hydrolysis for the precipitate with phosphotungstic acid (30·5 %) is due to the precipitation of polypeptides. This was evidenced also by the separation with the lysin of the body resembling the

* Journ. Expt. Med., ix., p.520, 1907.
† Zeit. physiol. Chem., lviii., S.224, 1908.

TABLE I.

	Hydrolysis I.		Hydrolysis II.	
	Nitrogen.	% of total Nitrogen.	Nitrogen.	% of total Nitrogen.
Nitrogen in hydrolysed fluid	2·15 gm.	100·00	12·32 gm.	100·00
Nitrogen as ammonia			1·05	8·52
Nitrogen as melanoidin			0·224	1·82
Nitrogen precipitated by phosphotungstic acid after removal of ammonia	0·656	30·50	2·14	17·36
Nitrogen precipitated by silver and baryta	0·238	11·07	1·045	8·48
Nitrogen in filtrate from silver and baryta (by difference)	0·418	19·43	1·095	8·88
Nitrogen in filtrate from silver and baryta, pptd. by phosphotungstic acid; this gives nitrogen as lysin...	0·147 (0·054)*	6·84 (2·51)	0·548	4·45
Nitrogen in the histidin portion	0·0293	1·37	0·16	1·30
Nitrogen in the arginin portion	0·117	5·44	0·685	5·56
Nitrogen pptd. by silver and baryta and not pptd. as histidin or arginin	0·038†	1·77	0·20†	1·62
Nitrogen in the filtrate from silver and baryta and not pptd. by phosphotungstic acid	—	—	0·19†	1·55

* Calculated from the separated lysin picrate.
† Amount actually estimated, the remainder unaccounted for.

dipeptide of Levene & Beatty.* Owing to this admixture the lysin nitrogen was calculated from the lysin picrate as 0·054 gm., and not from the nitrogen precipitated by the phosphotungstic acid, viz., 0·147 gm.

On the other hand, 17·36 % in the second hydrolysis is a low figure, since the method of Hausmann gives 21·33 %. Our low figure is due to the incomplete washing of the precipitate of barium sulphate and phosphotungstate.

The figures of histidin and arginin agree well. It must be pointed out that a considerable loss of nitrogen occurred with each precipitate of barium sulphate, with the result that the nitrogen figures do not add up. Thus, in experiment ii., 0·548 gm. nitrogen as lysin plus 0·19 gm. other than lysin, or 0·74 gm. nitrogen were obtained from 1·095 gm. nitrogen present originally.

The protein in the second hydrolysis amounted to 89·6 gm. The lysin calculated from the nitrogen present in the solution from which the lysin picrate was precipitated was 2·86 gm. The lysin picrate once recrystallized weighed 5·47 gm.

0·133 gm. lysin picrate gave 17·5 c.c. $\frac{N}{10}$ NH_3 by Kjeldahl-Jodlbauer-Gunning method.

$C_6H_{14}N_2O_2.C_6H_2(NO_2)_3OH$ requires 18·66 % N. Found 18·42 % N.

The solution of histidin contained nitrogen equal to 0·59 gm. histidin. There was crystallized 0·25 gm. histidin dichloride and 0·484 gm. picrolonate.

0·0618 gm. histidin dichloride gave 0·0762 gm. AgCl.

$C_6H_9N_3O_2.2HCl$ requires 31·18 % Cl. Found 30 5 % Cl.

It was noted that the histidin dichloride gave a slight residue of barium salt upon combustion.

The solution of arginin contained nitrogen equivalent to 2·14 gm. arginin. A portion was lost, but from a fraction containing 0·433 gm. nitrogen, 2·437 gm. arginin nitrate were obtained. The arginin nitrate was converted into arginin copper nitrate.

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* *Loc. cit.*

0·219 gm. arginin copper nitrate gave 0·02372 gm. Cu.

$(C_6H_{14}N_4O_2)_2.Cu(NO_3)_2.3H_2O$ requires 10·79% Cu. Found 10·83%.

0·219 gm. arginin copper nitrate gave 0·020 gm. H_2O.

$(C_6H_{14}N_4O_2)_2.Cu(NO_3)_2.3H_2O$ requires 9·16% H_2O. Found 9·14%.

The amounts of lysin, histidin, and arginin present in 100 gm. protein of egg-white are therefore—

Lysin	...	3·19%
Histidin	...	0·66%
Arginin	...	2·39%

In conclusion, we express our thanks to Professor Anderson Stuart, in whose laboratory this investigation was conducted.